

# **EXHIBIT B20**

## **Part 1**

## Personal Information

Black n' Red



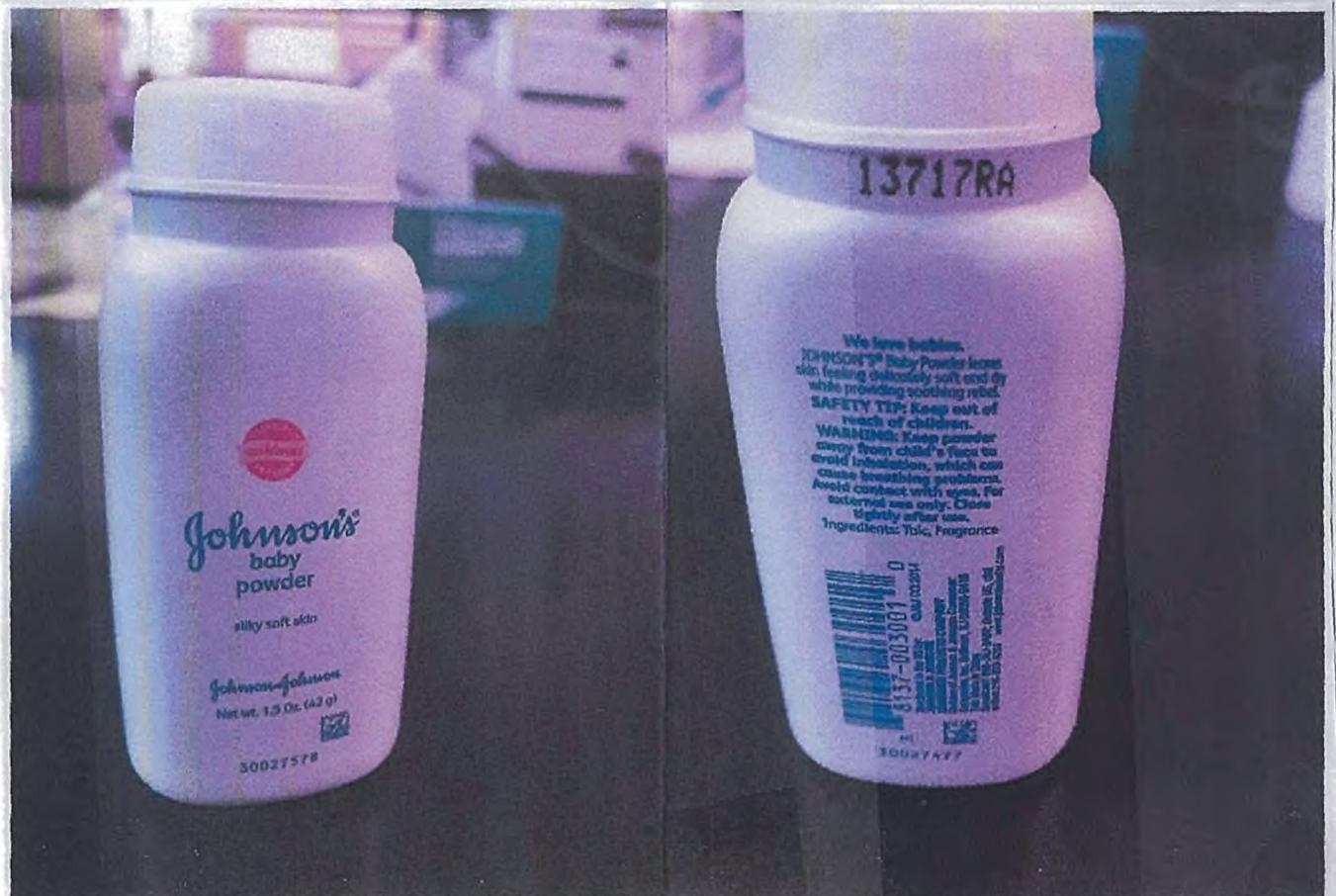
Talc / Baby Powder

10/15/17

Tried to dissolve talc (Fisher # T4-500 Lot #166820) vs  
Johnson + Johnson Baby Powder (# 30027477, Lot 13717RA)

- It won't completely dissolve - used DMSO & filtered
  - 50mg/ml
  - treated 200ug/ml or 500 ug/ml in dishes
  - 8ul for 200ug/ml
  - 20ul for 500ug/ml





11/13/17

Talc / Baby Powder Treated  
EOC, Normal Ov. Epi, Macrophages

- Split OV90 cells, 150mm dish x2
- Split macrophages (had  $4.8 \times 10^6$  cells in 150 mm plate)
- Split C.B. Normal ov. epi. cells
  - had 2 x 150mm dish  $\Rightarrow \sim 11 \times 10^6$  cells
  - split into 4 plates
- Seeded TOV112-D Unt. cells - treat after 24hr rest -  
(full plate was  $\sim 12 \times 10^6$  cells)

6 plates per timepoint in 100mm dish

<u>ID</u>	<u>treatment</u>	<u>details</u>
266	TOV112 - Unt-24 hr	$2 \times 10^6$ cells
267	Control (DMSO) - 24h	"
268	500 $\mu\text{g}/\text{mL}$ Talc - 24h	"
269	1000 $\mu\text{g}/\text{mL}$ Talc - 24h	"
270	500 $\mu\text{g}/\text{mL}$ Baby Powder (BP) - 24h	"
271	1000 $\mu\text{g}/\text{mL}$ BP - 24h	"
272	TOV112 - Unt 48 hr	$1 \times 10^6$ cells
273	Control 48h	"
274	500 $\mu\text{g}/\text{mL}$ Talc 48h	"
275	1000 $\mu\text{g}/\text{mL}$ Talc 48h	"
276	500 $\mu\text{g}/\text{mL}$ B.P. - 48h	"
277	1000 $\mu\text{g}/\text{mL}$ B.P. - 48h	"
278	TOV112D - unt 72 hr	500,000 cells
279	Control - 72hr	"
280	500 $\mu\text{g}/\text{mL}$ Talc - 72h	"
281	1000 $\mu\text{g}/\text{mL}$ Talc - 72h	"
282	500 $\mu\text{g}/\text{mL}$ B.P. 72h	"
283	1000 $\mu\text{g}/\text{mL}$ B.P. 72hr	"

from pg 2

11/14/17

Treat cells after 24 hr rest

$$(x)(50,000 \mu\text{g/ml}) = (10\text{ml})(500 \mu\text{g/ml})$$

$$x = 100 \mu\text{l}$$

$$\text{for } 1000 \mu\text{g/ml} = 200 \mu\text{l}$$

Controls get 200μl of sterile Dmso

- made a master mix of media + treatment and then added it to the cells

$$500 \mu\text{g/ml} = 300 \mu\text{l} + 30 \text{ ml}$$

$$1000 \mu\text{g/ml} = 600 \mu\text{l} + 30 \text{ ml}$$

$$\text{Dmso Control} = 600 \mu\text{l} + 30 \text{ ml}$$

<u>ID</u>	<u>Treatment</u>	<u>ID</u>	<u>Treatment</u>
284	OV90 Untreated 24 hours	320	TOV-21G Untreated 24 hours
285	24 hr DMSO Control	321	24 hr DMSO Control
286	24 hr 500 ug/ml Talc	322	24 hr 500 ug/ml Talc
287	25 hr 1000 ug/ml Talc	323	25 hr 1000 ug/ml Talc
288	24 hr 500 ug/ml Baby Powder	324	24 hr 500 ug/ml Baby Powder
289	24 hr 1000 ug/ml Baby Powder	325	24 hr 1000 ug/ml Baby Powder
290	Ov90 Untreated 48 hours	326	TOV-21G Untreated 48 hours
291	48 hr DMSO Control	327	48 hr DMSO Control
292	48 hr 500 ug/ml Talc	328	48 hr 500 ug/ml Talc
293	48 hr 1000 ug/ml Talc	329	48 hr 1000 ug/ml Talc
294	48 hr 500 ug/ml Baby Powder	330	48 hr 500 ug/ml Baby Powder
295	48 hr 1000 ug/ml Baby Powder	331	48 hr 1000 ug/ml Baby Powder
296	Ov90 72 hr untreated	332	TOV-21G 72 hr untreated
297	72 hr DMSO Control	333	72 hr DMSO Control
298	72 hr 500 ug/ml Talc	334	72 hr 500 ug/ml Talc
299	72 hr 1000 ug/ml Talc	335	72 hr 1000 ug/ml Talc
300	72 hr 500 ug/ml Baby Powder	336	72 hr 500 ug/ml Baby Powder
301	72 hr 1000 ug/ml Baby Powder	337	72 hr 1000 ug/ml Baby Powder
302	EL1 Untreated 24 hours	338	Cell Biologics - Normal Ovarian Epithelial
303	24 hr DMSO Control	339	cells, Unt 24 hrs
304	24 hr 500 ug/ml Talc	340	24 hr DMSO Control
305	25 hr 1000 ug/ml Talc	341	24 hr 500 ug/ml Talc
306	24 hr 500 ug/ml Baby Powder	342	25 hr 1000 ug/ml Talc
307	24 hr 1000 ug/ml Baby Powder	343	24 hr 500 ug/ml Baby Powder
308	EL1 Untreated 48 hours	344	24 hr 1000 ug/ml Baby Powder
309	48 hr DMSO Control	345	Cell Biologics - Normal Ovarian Epithelial
310	48 hr 500 ug/ml Talc	346	cells, Unt 48 hrs
311	48 hr 1000 ug/ml Talc	347	48 hr DMSO Control
312	48 hr 500 ug/ml Baby Powder	348	48 hr 500 ug/ml Talc
313	48 hr 1000 ug/ml Baby Powder	349	48 hr 1000 ug/ml Baby Powder
314	EL1 72 hr untreated	350	Cell Biologics - Normal Ovarian Epithelial
315	72 hr DMSO Control	351	cells, Unt 72 hrs
316	72 hr 500 ug/ml Talc	352	72 hr DMSO Control
317	72 hr 1000 ug/ml Talc	353	72 hr 500 ug/ml Talc
318	72 hr 500 ug/ml Baby Powder	354	72 hr 1000 ug/ml Baby Powder
319	72 hr 1000 ug/ml Baby Powder	355	72 hr 500 ug/ml Baby Powder

11/17/17

seeded C.B. Normal OV. Epi Cells

338-355 - passage 11

24h =  $2 \times 10^6$  cells

48h =  $1 \times 10^6$  cells

72h = 500,000 cells

will treat on 11/19/17

collected 24hr 11/20/17

48hr 11/21/17

72h 11/22/17

11/19/17 seeded EL-1, treated on 11/19/17

for 24hr  $4 \times 10^6$  collect 11/20/17

48hr  $2 \times 10^6$  collect 11/21/17

72hr  $1 \times 10^6$  collect 11/22/17

12/4/17 Seeded TAN120 / TAN21G / treat 12/5/17

collect 24hr 12/6/17

" 48hr 12/7/17

" 72hr 12/8/17

12/7/17

## RNA extraction

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
1	267	12/7/2017 2:07:20 PM	0.0635	µg/µl	1.588	0.831	1.91	1.36
2	269	12/7/2017 2:07:45 PM	0.0548	µg/µl	1.370	0.695	1.97	0.29
3	273	12/7/2017 2:08:08 PM	0.0257	µg/µl	0.643	0.335	1.92	0.24
4	275	12/7/2017 2:08:29 PM	0.0164	µg/µl	0.409	0.212	1.93	0.66
5	285	12/7/2017 2:08:53 PM	0.0678	µg/µl	1.694	0.882	1.92	1.13
6	287	12/7/2017 2:09:13 PM	0.0553	µg/µl	1.381	0.722	1.91	2.32
7	291	12/7/2017 2:09:32 PM	0.0630	µg/µl	1.575	0.802	1.96	0.34
8	293	12/7/2017 2:09:51 PM	0.0506	µg/µl	1.265	0.648	1.95	1.39
9	297	12/7/2017 2:10:10 PM	0.0358	µg/µl	0.896	0.455	1.97	0.22
10	299	12/7/2017 2:10:30 PM	0.0248	µg/µl	0.621	0.313	1.99	0.86
11	303	12/7/2017 2:10:51 PM	0.1809	µg/µl	4.523	2.334	1.94	1.35
12	305	12/7/2017 2:11:10 PM	0.1508	µg/µl	3.770	1.925	1.96	1.75
13	309	12/7/2017 2:11:31 PM	0.0279	µg/µl	0.698	0.362	1.93	0.85
14	311	12/7/2017 2:11:53 PM	0.0675	µg/µl	1.688	0.877	1.92	0.35
15	315	12/7/2017 2:12:12 PM	0.0445	µg/µl	1.113	0.585	1.90	1.13
16	317	12/7/2017 2:12:31 PM	0.0587	µg/µl	1.468	0.770	1.91	0.60
17	321	12/7/2017 2:12:50 PM	0.0810	µg/µl	2.025	1.061	1.91	1.03
18	323	12/7/2017 2:13:10 PM	0.0326	µg/µl	0.815	0.408	2.00	1.00
19	327	12/7/2017 2:13:31 PM	0.0445	µg/µl	1.112	0.574	1.94	2.54
20	329	12/7/2017 2:14:02 PM	0.0092	µg/µl	0.230	0.114	2.02	0.10
21	339	12/7/2017 2:14:21 PM	0.0177	µg/µl	0.442	0.220	2.01	0.55
22	341	12/7/2017 2:14:40 PM	0.0172	µg/µl	0.429	0.221	1.94	0.89
23	345	12/7/2017 2:14:59 PM	0.0219	µg/µl	0.548	0.281	1.95	1.31
24	347	12/7/2017 2:15:17 PM	0.0165	µg/µl	0.414	0.207	2.00	0.56
25	351	12/7/2017 2:15:34 PM	0.0165	µg/µl	0.413	0.214	1.93	0.96
26	353	12/7/2017 2:15:52 PM	0.0112	µg/µl	0.281	0.142	1.98	0.94
27	279	12/8/2017 1:19:05 PM	0.0145	µg/µl	0.364	0.192	1.89	1.07
28	281	12/8/2017 1:19:28 PM	0.0089	µg/µl	0.222	0.111	2.00	0.48
29	333	12/8/2017 1:19:56 PM	0.0244	µg/µl	0.609	0.317	1.92	0.65
30	335	12/8/2017 1:20:15 PM	0.0039	µg/µl	0.097	0.054	1.79	0.39
31	335	12/8/2017 1:21:01 PM	0.0041	µg/µl	0.102	0.048	2.12	0.38

12/8/17

## (V10) CONA synthesis

0.1 μg RNA used except  
for #335 (0.06 μg)

Sample ID	μl RNA for 0.1 μg rxn	μl Water
267	1.6	14.4
269	1.8	14.2
273	3.9	12.1
275	6.1	9.9
279	6.9	9.1
281	11.2	4.8
285	1.5	14.5
287	1.8	14.2
291	1.6	14.4
293	2.0	14.0
297	2.8	13.2
299	4.0	12.0
303	0.6	15.4
305	0.7	15.3
309	3.6	12.4
311	1.5	14.5
315	2.2	13.8
317	1.7	14.3
321	1.2	14.8
323	3.1	12.9
327	2.2	13.8
329	10.9	5.1
333	4.1	11.9
335	16.0	0.0
339	5.6	10.4
341	5.8	10.2
345	4.6	11.4
347	6.1	9.9
351	6.1	9.9
353	8.9	7.1

1/10/18

CA-125 EUA

RayBio # ELH-CA125

Test unconcentrated media vs concentrated using Amicin  
Ultra-15 filter MW cutoff 10,000

<u>Weigh tubes</u>	<u>Volume empty</u>	<u>empty sample reservoir</u>	<u>empty centrifuge tube</u>	<u>medium reservoir</u>
		<u>w/ media</u>		
266	8.36mL 25.0g	10.99g	11.59g	19.17g
338	8.853mL 24.9g	10.98g	11.53g	19.7g

Spin tubes for 25 min at 4000xg

move the retentate by pipetting into new container

<u>weigh retentate</u>	<u>tube weight</u>	<u>tube + retentate</u>	<u>filtrate</u>
266	1.0025g	1.3514g	19.29g
338	1.0088g	1.7104g	19.4797g

$$\% \text{ retentate recovery} = 100 \times \frac{W_r \times C_r}{W_0 \times C_0}$$

$$\% \text{ filtrate recovery} = 100 \times \frac{W_f \times C_f}{W_0 \times C_0}$$

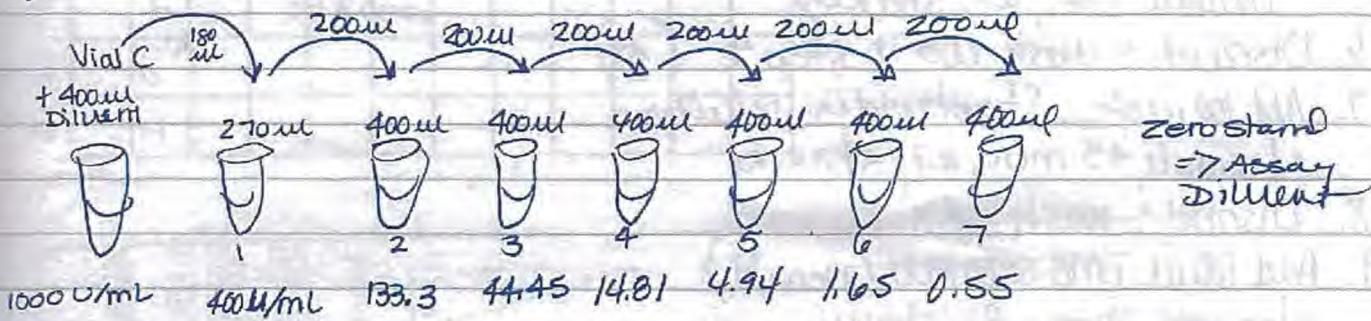
$$\% \text{ recovery} = \% \text{ retentate recovery} + \% \text{ filtrate recovery}$$

1/10/18

OA-125 EUST

41040

1. All reagents & samples to room temperature
2. Assay diluent (Item E2) Should be diluted 5x w/ dd H<sub>2</sub>O
  - Stable 1 mo. at 4°C
3. Prep. of Standard
  - Spin vial C
  - Add 400 μL 1x Assay Diluent into vial C = 1000 U/mL
    - mix gently
  -



4. Prepare Wash buffer by diluting 20x
  - Stable 1 mo. at 4°C

5. Spin Item F, detection antibody
  - Add 100 μL of 1X Assay Diluent
    - Stable 5 days at 4°C
  - Dilute it <sup>80x</sup> ~~80x~~ <sup>1:800</sup> <sub>assay diluent</sub> and will be used in assay

6. Spin HRP-Strep. Vial (Item G) & mix
  - Add 15 μL to tube w/ 12 mL of assay diluent (800x)
    - do not save!

Go to pg 10

### Assay Procedure

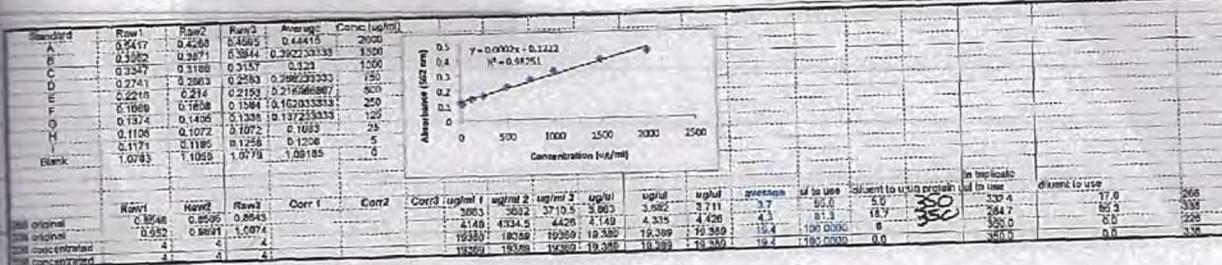
1. All samples to RT.
2. Label 8 strip/wells
3. Add 100 $\mu$ l Standard /Samples
  - Incubate 2.5 hr, gentle shaking, RT
4. Discard solution, wash 4x
  - decant, blot after washes
5. Add 100 $\mu$ l of 1X Antibody mix
  - Incubate 1 hr, RT, shaking
6. Discard & wash as in step 4 (4x)
7. Add 100 $\mu$ l of Streptavidin solution
  - Incubate 45 min, RT, shaking
8. Discard & wash 4x
9. Add 100 $\mu$ l TMB substrate (Item H)
  - Incubate 30 min, RT, shaking
10. Add 50 $\mu$ l STOP solution (Item I) to each well
  - Read at 450nm

41042

1/10/18

41042

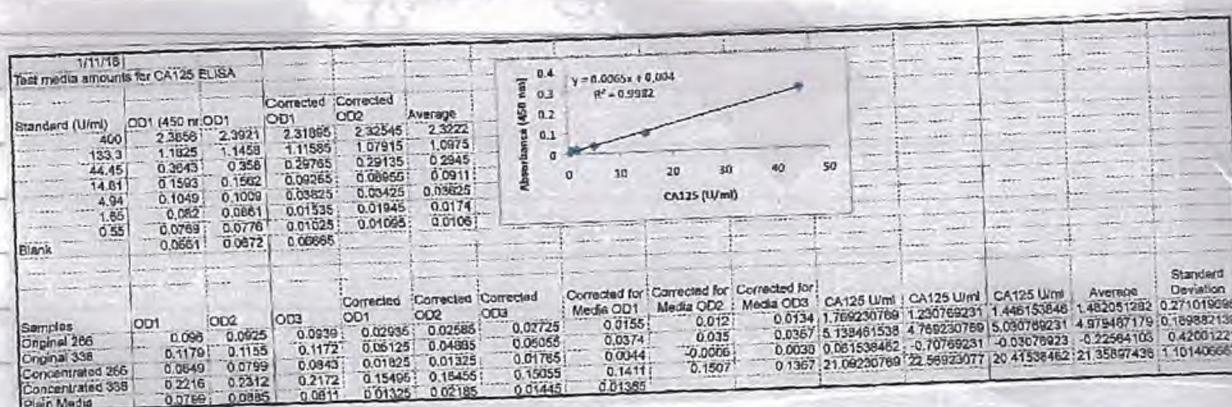
## Protein levels in media for CH12S Assay



1/11/18

## CA125 ELISA - test levels in media

	1	2	3	4	5	6	7	8	9	10	11	12
A	Stand	1		266 orig								
B		2		338 orig.								
C		3		266 conc								
D		4		338 conc								
E		5		media blank								
F		6										
G		7										
H	Blank											



266 = Tumour unit

338 = Normal Ov. Epi. Cells

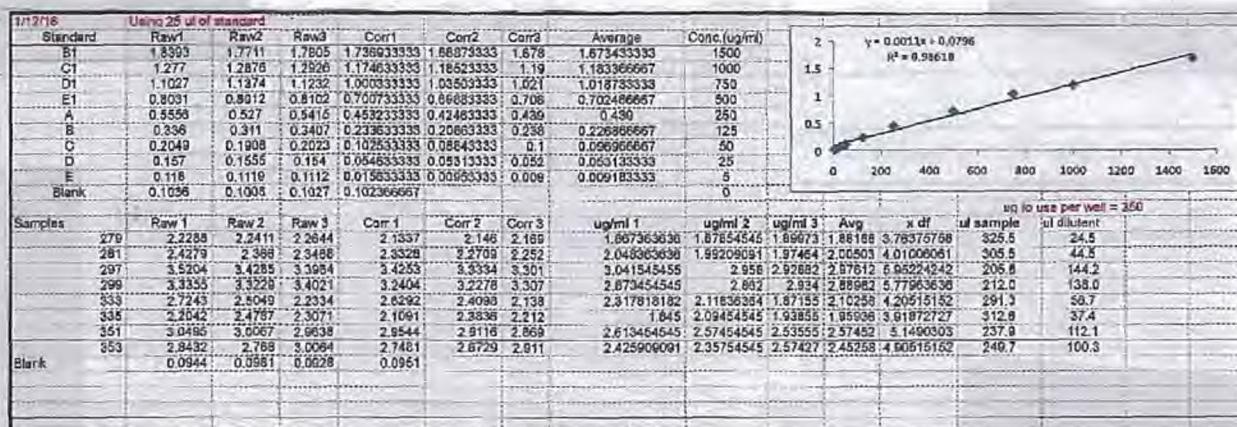
Proceed using unconcentrated media

1/12/18

## Protein levels for CA125 assay

Re-did standard and original media - media was too concentrated

- diluted media by 50%, remeasured
- Also used 25  $\mu$ l of the standard and samples



1/12/2018

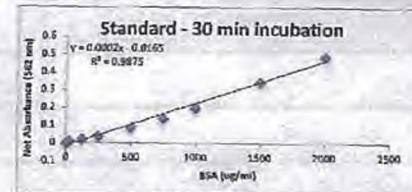
Samples	OD1	OD2	OD3	Corrected	Corrected	Corrected	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
				OD1	OD2	OD3					
TOV112D 72 hr control	279	0.104	0.0885	0.0069	0.03735	0.02186	0.03025	6.13076923	2.74615385	4.03846154	4.58401638
TOV112D 72 hr 1000 ug/ml Talc	281	0.006	0.0742	0.0638	0.01305	0.00765	0.01715	1.63076923	0.54615385	2.02307692	0.77237818
Ov90 72 hr control	297	0.1059	0.1046	0.1321	0.03925	0.03795	0.08545	5.42307692	9.45384615	5.32307692	0.34811411
Ov90 72 hr 1000 ug/ml Talc	299	0.1062	0.0908	0.1316	0.03955	0.02985	0.05495	5.46923077	3.06923077	8.37692308	4.28923077
TOV-21G 72 hr control	333	0.0724	0.0716	0.0942	0.00675	0.00496	0.02765	0.265923077	0.14615385	3.62307692	0.69705927
TOV-21G 72 hr 1000 ug/ml Talc	335	0.0761	0.077	0.0956	0.00945	0.01035	0.01885	0.63846154	0.97692308	2.3	0.90709231
Normal Ov Epithelial 72 hr control	351	0.1003	0.0943	0.1001	0.03365	0.02765	0.03345	4.58153845	3.53946154	4.53076923	4.54615385
Normal Ov Epithelial 72 hr 1000 ug/ml Talc	363	0.1106	0.0892	0.1331	0.04395	0.02535	0.06545	6.14615385	3.28461538	9.80769231	4.71538462
	b	0.0661	0.0672	0.06665							2.02341325

The other proteins in media may be interfering. Try lysate.

1-16-18

## Protein levels in lysate

Standard ID	Concentration n (ug/ml)	OD1	OD2	OD3	Average	Corrected Avg
A	2000	0.5868	0.5848	0.5862	0.5796667	0.4874
B	1500	0.4487	0.4211	0.3533	0.4334	0.34463335
C	1000	0.3213	0.2774	0.2593	0.286	0.19743335
D	750	0.2473	0.2199	0.2128	0.2286667	0.1391
E	500	0.181	0.1759	0.1753	0.1766667	0.0875
F	250	0.1322	0.1252	0.1256	0.1276667	0.0363
G	125	0.1105	0.112	0.1116	0.1113667	0.0228
H	25	0.1004	0.0841	0.0953	0.0869	0.0803333
I	5	0.0545	0.0640	0.0941	0.0877333	0
J (BLANK)	0	0.0681	0.0884	0.0802	0.0856667	0



	ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ml 1	ug/ml 2	ug/ml 3	Average	using 100 ug per well	x 3.5 wells	diluent
TOV112D 72 hr control	279	0.2429	0.2301	0.2326	0.1358	0.1226	0.1253	7.605	8.095	7.09	7.0275	14.23	49.9	302.2
TOV112D 72 hr 1000 ug/ml Talc	281	0.1862	0.1923	0.1578	0.0818	0.085	0.0905	4.92	5.076	4.85	4.9433333	20.21	70.7	278.3
Ov90 72 hr control	297	0.3108	0.331	0.3008	0.2125	0.2237	0.2233	11.46	12.01	11.98	12	8.33	29.2	300.8
Ov90 72 hr 1000 ug/ml Talc	298	0.2659	0.2759	0.2784	0.1816	0.1866	0.1711	8.805	9.255	8.38	8.975	10.72	37.6	312.4
TOV-21G 72 hr control	333	0.2961	0.2988	0.2948	0.1918	0.1895	0.1875	10.415	10.3	10.2	10.305	8.76	34.0	316.0
TOV-21G 72 hr 1000 ug/ml Talc	335	0.1053	0.1616	0.1034	0.0845	0.0543	0.0901	3.75	3.54	3.63	3.84	27.47	96.2	253.8
Normal Ov Epithelial 72 control	351	0.2359	0.23	0.23	0.1208	0.1227	0.1227	7.255	6.98	6.96	6.96	14.37	50.3	299.7
Normal Ov Epithelial 72 hr 1000 ug/ml talc	a	0.2432	0.2474	0.2344	0.1359	0.1401	0.1271	7.82	7.83	7.18	7.725	12.94	45.3	304.7
TOV112D 72 hr unk	278	0.6686	0.6925	0.585	0.5646667	0.5837667	0.5492667	29.063333	30.263333	28.2845333	28.2778333	5.42	12.0	338.0
Ov90 72 hr unk 72 hr	296	0.34	0.3407	0.3206	0.2362667	0.2369667	0.2308667	12.538333	12.673333	12.4683333	12.5933333	7.94	27.8	322.2
TOV-121G unk	332	0.4382	0.4456	0.4413	0.3546667	0.3410667	0.3375667	17.568333	17.618333	17.703333	17.5103333	5.61	18.7	353.3
Normal Ov Epithelial 72 hr unk	b	0.2281	0.2247	0.2216	0.1243667	0.1309667	0.1278667	7.0433333	7.0733333	7.2103333	7.2058333	13.71	48.0	302.0

	1	2	3	4	5	6	7	8	9	10	11	12
A									279			
B									281			
C									297			
D									299			
E									333			
F									335			
G									335			
H									351			

	1	2	3	4	5	6	7	8	9	10
A	Stund	A	I							
B	B		PBS Blank							
C	C		278							
D	D		296							
E	E		332							
F	F		350							
G	G		Lysis Buffer							
H	H									

CA125 in lysate

	1	2	3	4	5	6	7	8	9	10	11	12
A	279											
B	281											
C	297											
D	299											
E	333											
F	335											
G	351											
H	353											

	1	2	3	4	5	6	7	8	9	10	11	12
A												278
B												278
C												296
D												296
E												332
F												332
G												350
H												350

1/17/2018	Lysate	Standard Deviation											
		Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
TOV-112D 72 hr control		279	0.1637	0.1749	0.171	0.0907	0.0919	0.098	11.8	13.5230769	12.6230769	13.2230769	0.42426407
TOV-112D 72 hr 1000 ug/ml Talc		281	0.1165	0.1175	0.1221	0.0358	0.0345	0.0361	4.89230769	4.89230769	5.4	4.95487179	0.36482413
Ov90 72 hr control		297	0.146	0.1479	0.189	0.063	0.0649	0.0893	0.07682308	9.36923077	12.8	9.2230769	0.20689275
Ov90 72 hr 1000 ug/ml Talc		299	0.1371	0.13	0.1506	0.0541	0.047	0.0776	7.70768231	6.61538462	9.78461538	7.16153646	0.77237818
TOV-21G 72 hr control		333	0.1374	0.1269	0.1269	0.0544	0.0439	0.0469	7.75384615	6.13846154	8.44615385	6.20230769	0.21751132
TOV-21G 72 hr 1000 ug/ml Talc		335	0.1491	0.1518	0.1547	0.0661	0.0686	0.0777	2	0.29230769	0.56923077	0.40768923	0.19581419
Normal Ov Epithelial 72 hr control		351	0.1467	0.1467	0.0721	0.0532	0.0637	0.0717	9.56384615	9.80623077	10.4153845	9.78153845	0.20372128
Normal Ov Epithelial 72 hr 1000 ug/ml Talc	b	353	0.1551	0.1382	0.1467	0.0721	0.0632	0.0637	10.4769231	7.58923077	9.18461538	9.83076923	0.91378853

1/18/2018	Lysate	Standard Deviation											
		Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
TOV-112D 72 hrunt		278	0.1698	0.1904	0.1904	0.0476	0.0332	0.0476	5.70768231	5.26153848	5.98461538	5.02258519	
Ov90 72 hrunt 72 hr		296	0.1978	0.1971	0.1971	0	0.0466	0.0449	0.4	6.29230788	6.24815385	6.07614996	
TOV-21G 72 hrunt		332	0.1875	0.179	0.199	0.0353	0.0286	0.0468	4.81538462	3.00768231	6.58461538	4.16153646	0.9246781
Normal Ov Epithelial 72 hrunt	b	350	0.2533	0.2874	0.2328	0.1011	0.1352	0.0805	14.8384615	20.1846154	11.7646154	13.3615385	2.230106

Results: Lysate protein measurement may be affected by talc.  
 Repeat protein Measurements, have control w/ talc in it.

Re-measured protein levels in media 41047

1-19-18

• Recalc. data. CA125

	1	2	3	4	5	6	7	8	9	10	11	12
A				279								
B				281								
C				297								
D				299								
E				333								
F				335								
G				351								
H				353								

Re-measure media  
protein

1-19-18

- 10x diluted samples
- media was 10x as blank
- used 75µl to detect

protein → New control

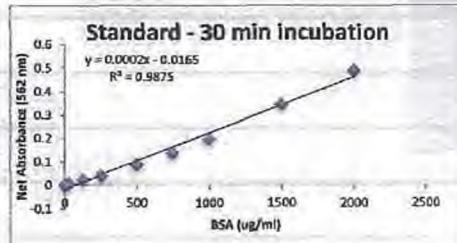
	1	2	3	4	5	6	7	8	9	10	11	12
A					278	335						
B					279	350						
C					281	351						
D					296	353						
E					297	Lysis Buffer						
F					299	Lysis Buffer + talk						
G					332							
H					333							

$$44 \times 200 = \frac{8800}{50} = 176$$

Go to pg 17

from pg 1b

Standard ID	Concentration ( $\mu\text{g/ml}$ )	Corrected				
		OD1	OD2	OD3	Average	Avg
A	2000	0.5869	0.5848	0.5562	0.575967	0.4874
B	1600	0.4457	0.4211	0.3533	0.4334	0.344833
C	1000	0.3213	0.2774	0.2593	0.286	0.197433
D	750	0.2473	0.2199	0.2128	0.226687	0.1391
E	500	0.181	0.1759	0.1713	0.176067	0.0875
F	250	0.1328	0.1252	0.1258	0.127887	0.0393
G	125	0.1105	0.112	0.1116	0.111367	0.0228
H	25	0.1004	0.0941	0.0953	0.0966	0.008033
I	5	0.0845	0.0846	0.0841	0.087733	0
J (BLANK)	0	0.0881	0.0884	0.0892	0.088567	0



ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ml 1	ug/ml 2	ug/ml 3	Average	how much was used	ug used
TOV112D 72 hr unt	278	0.6692	0.6792	0.6667	0.559657	0.6792	0.6667	28.81833	34.785	34.16	34.4725	3.42
TOV112D 72 hr control	279	0.2121	0.2206	0.2113	0.102767	0.2206	0.2113	5.963333	11.855	11.39	11.6225	14.23
TOV112D 72 hr-1000 ug/ml Talc	281	0.1886	0.1902	0.189	0.077267	0.1902	0.189	4.688333	10.335	10.275	8.432778	20.21
Ov90 72 hr unt 72 hr	296	0.3372	0.3405	0.3354	0.227867	0.3405	0.3354	12.21833	17.85	17.595	15.88778	7.94
Ov90 72 hr control	297	0.3217	0.3231	0.3255	0.212367	0.3231	0.3255	11.44333	16.98	17.1	17.04	8.33
Ov90 72 hr 1000 ug/ml Talc	299	0.2813	0.282	0.2785	0.171967	0.282	0.2785	9.423333	14.925	14.75	14.8375	10.73
TOV-12G unt	332	0.4161	0.4393	0.452	0.306767	0.4393	0.452	16.16333	22.79	23.425	23.1075	5.61
TOV-21G 72 hr control	533	0.3031	0.3039	0.3238	0.193767	0.3039	0.3238	10.51333	16.02	17.015	14.51611	9.70
Normal Ov Epithelial 72 hr unt	335	0.1847	0.1856	0.18	0.075367	0.1856	0.18	4.583333	10.105	10.325	8.341111	27.47
Normal Ov Epithelial 72 hr control	350	0.2227	0.2338	0.2367	0.113367	0.2338	0.2367	6.493333	12.515	12.66	12.5875	13.71
Normal Ov Epithelial 72 hr 1000 ug/ml talc	351	0.2287	0.2316	0.2402	0.119367	0.2316	0.2402	5.793333	12.405	12.835	12.82	14.37
Lysis buffer blank	353	0.2277	0.2391	0.2432	0.118367	0.2391	0.2432	6.743333	12.78	12.985	9.761667	12.94
Lysis buffer+talc blank		0.1102	0.1083	0.1095	0.109333							
		0.1073	0.1068	0.1059	-0.00203	-0.00253	-0.00343	-0.00267				

Remeasured Media  
Protein - 10x diluted  
using 25ul standard

Samples	Raw 1	Raw 2	Raw 3	Corr 1	Corr 2	Corr 3	ug/ml 1	ug/ml 2	ug/ml 3	Avg	xdf	ul media	actual ug used
279	0.7155	0.7569	0.7571	0.8192	0.6608	0.6808	0.490545	0.528182	0.528364	0.515687	5.15897	92.992174	479.5578
281	0.7067	0.7139	0.7355	0.6104	0.6176	0.6393	0.482545	0.489091	0.508818	0.493485	4.934848	87.280476	430.7159
297	1.1257	1.1418	1.1655	1.0294	1.0455	1.0661	0.883455	0.878091	0.899727	0.880424	6.804242	58.801368	517.7015
299	1.1303	1.1325	1.1842	1.034	1.0362	1.0879	0.857636	0.859836	0.916638	0.884636	6.845364	60.557443	535.7132
333	0.7006	0.7217	0.7401	0.6043	0.6254	0.6438	0.477	0.496182	0.512909	0.495364	4.953656	63.231246	412.2973
335	0.7315	0.7361	0.7684	0.6352	0.6398	0.6721	0.505091	0.508273	0.538836	0.517667	89.314713	462.3525	
351	0.9217	0.9433	0.9655	0.8254	0.8467	0.8692	0.578	0.697368	0.717818	0.697816	6.978182	67.973964	474.3347
353	0.9401	0.9552	1.0083	0.8438	0.8689	0.912	0.684727	0.744818	0.756727	0.7320909	7.320909	71.353555	522.3729
Blank-10x media	0.7339	0.7117	0.7024	0.6376	0.7117	0.7024	0.70705						
Blank-PBS	0.0941	0.0998	0.095	0.0963									

Recalculated how much protein was really used  
and then adjusted CA125 levels pg 18

from pg 17

## CA125 -recalc. for SRI abstract

1/12/2018																			
Sample	OD1			OD2			OD3			Corrected OD1			Corrected OD2			Corrected OD3			CA125 Unit
	CA125 Unit	CA125 Unit	CA125 Unit	Average	Standard Deviation	Correlated per ug protein	Correlated per ug protein	Correlated per ug protein	Average	SD	protein	protein	protein	protein	protein	protein	protein	protein	
TOV112D 72 hr control	278	8.154	0.0486	0.0200	0.0175	0.0195	0.0228	0.0228	8.12079322	2.7401835	4.53040154	4.55481538	8.77227816	0.0166893	0.00572543	0.00542122	Average	SD	
TOV112D 72 hr 1000 ug/ml Talc	281	5.000	0.0743	0.0200	0.0175	0.0195	0.0228	0.0228	5.12079322	2.7401835	4.53040154	4.55481538	8.77227816	0.0166893	0.00572543	0.00542122	0.00787	0.00191	
CV99 72 hr control	297	8.159	0.1046	0.1321	0.0923	0.0764	0.0845	0.0845	5.42307692	8.42304815	8.22377692	8.42304815	8.1145144	0.1197165	0.0116165	0.0116165	0.00116	0.00017	
CV99 72 hr 1000 ug/ml Talc	298	8.1172	0.0960	0.1318	0.0935	0.0828	0.0986	0.0986	5.4697635	2.06923771	5.2785233	4.36810077	8.1414236	0.0166893	0.00572543	0.00542122	0.0116165	0.00017	
TOV210 72 hr control	333	8.3724	0.0798	0.0942	0.0535	0.0648	0.0275	0.0275	5.38120207	3.02507852	4.36810077	4.36810077	8.1145144	0.0054013	0.0185237	0.0185237	0.001548	0.00015	
CV99 72 hr 1000 ug/ml Talc	335	8.191	0.0777	0.0906	0.0525	0.0625	0.0188	0.0188	5.0340164	0.97693308	2.3	0.8670831	0.97309799	0.00555141	0.0060477	0.00755524	0.00643	0.00015	
Normal Ov Epithelial 72 hr control	331	8.1592	0.0631	0.1001	0.0420	0.0265	0.0394	0.0394	4.30192448	2.0240154	4.30192448	4.30192448	8.0219197	0.00203713	0.00476658	0.001189	0.000223	0.000223	
Normal Ov Epithelial 72 hr 1000 ug/ml Talc	323	8.1108	0.0822	0.1191	0.0493	0.0223	0.0541	0.0541	5.14610385	3.25491508	9.46702321	4.71238462	2.23341325	0.01281629	0.00694258	0.00203448	0.01143	0.00015	
b	8.0961	0.0672	0.0665																

1/12/2018																			
Sample	OD1			OD2			OD3			Corrected OD1			Corrected OD2			Corrected OD3			CA125 Unit
	CA125 Unit	CA125 Unit	CA125 Unit	Average	Standard Deviation	Correlated per ug protein	Correlated per ug protein	Correlated per ug protein	Average	SD	protein	protein	protein	protein	protein	protein	protein	protein	
TOV112D 72 hr control	278	8.166	0.0564	0.0194	0.0175	0.0195	0.0228	0.0228	5.20758231	6.29160948	3.36481524	3.36481524	3.02228518	0.02086602	0.04620356	0.02050	0.00887	0.00015	
TOV112D 72 hr 1000 ug/ml Talc	279	8.167	0.1246	0.171	0.0923	0.0828	0.0986	0.0986	11.8	12.022765	7.22303769	6.42452127	6.42452127	6.07113785	0.0166893	0.00572543	0.00542122	0.00015	0.00015
CV99 72 hr 1000 ug/ml Talc	281	8.1188	0.1175	0.1321	0.0938	0.0645	0.0391	0.0391	4.89203705	4.90913065	5.4	4.89203705	4.89203705	4.05199522	0.0166893	0.00572543	0.00542122	0.00015	0.00015
CV99 72 hr control	295	8.1878	0.1971	0	0.0493	0.0449	0.0649	0.0649	8.076322006	6.20227693	6.54151905	6.54151905	6.07634979	0.02072387	0.04039765	0.02050	0.008206	0.00015	
CV99 72 hr 1000 ug/ml Talc	297	8.148	0.1478	0.1628	0.093	0.0649	0.0808	0.0808	8.076322006	6.30623177	12.8	9.23307662	9.26608775	0.0354757	0.0060099	0.0887878	0.02030	0.00115	
CV99 72 hr control	299	8.1371	0.13	0.1598	0.0561	0.0247	0.047	0.047	7.7676231	6.81259462	9.79461538	7.81102645	8.77227816	0.04185225	0.08446066	0.02095	0.0101	0.00015	
TOV210 72 hr control	315	8.1579	0.1159	0.1628	0.0923	0.0648	0.0808	0.0808	4.15133945	5.12052015	6.41513394	6.41513394	6.1815211	0.02086602	0.04620356	0.02050	0.00871	0.00015	
TOV210 72 hr 1000 ug/ml Talc	323	8.1574	0.1569	0.1295	0.0544	0.0436	0.0458	0.0458	7.73334019	1.7849154	6.64613285	6.64613285	4.91524548	0.02072387	0.04039765	0.02072387	0.00015	0.00015	
Normal Ov Epithelial 72 hr control	325	0.1	0.0269	0.0507	0.017	0.0539	0.0277	0.0277	2	2.0237706	0.5952627	6.43075525	5.1755145	5.00572388	0.01137673	0.0246411	0.00492	0.00015	0.00015
Normal Ov Epithelial 72 hr 1000 ug/ml Talc	326	0.233	0.204	0.2258	0.1011	0.1332	0.0806	0.0806	14.9354615	23.348415	11.7345154	13.2610335	12.2361035	0.66559233	0.11665195	0.03282767	0.0774	0.0122	
Normal Ov Epithelial 72 hr control	321	0.1491	0.1516	0.1547	0.0001	0.0682	0.0717	0.0717	9.35334615	8.96833077	16.4153846	16.4153846	8.73372128	0.02558195	0.0247735	0.07432668	0.0598	0.00115	
Normal Ov Epithelial 72 hr 1000 ug/ml Talc	323	0.1591	0.1552	0.1467	0.0271	0.0332	0.0637	0.0637	10.4799531	7.58823077	9.18451532	8.83076823	9.31799581	0.02602299	0.07271144	0.077103	0.00015	0.00015	
b	8.0956	0.0676	0.0748	0.005															

fold increase fold increase fold increase Average SD

OV90	1.02745665	0.5765896	1.76156069	1.39450867	0.51908995
TOV21A	4.03703704	4.7037037	11.0740741	4.37037037	0.47140452
	↳ Normal Ov Epi				
	1.35194585	0.72250423	2.11336717	1.73265651	0.53840618

Media calculations for OV90, 21A, Normal Ov. Epi were used after SRI abstract

Seed 72 hour 1000 µg/ml Talc EOC

SKW-3 and A2780 seeded  $4 \times 10^6$  cells

Tov-21G seeded  $2 \times 10^6$  cells

Treat SKW-3 and A2780 today 1/29/18

25ml of media

$$(x)(100\text{mg/ml}) = (25\text{ml})(1000\text{\mu g/ml})$$
$$x = 250\text{ml}$$

Treat Tov21G 1/30/18

1/31/18 - The presence of 1000 µg/ml is physically killing the cells.

- We need to decrease dose.

1/3 2/11/18

Treat EOC w/talc

- Needed to lower the dose of talc (in saline) <sup>PBS</sup>
- Unt, 5, 20, 100  $\mu\text{g}/\text{mL}$   $\rightarrow$  72 hours

- Seeded  $1 \times 10^6$  cells 60mm dish, 5mL

- ELI Unt 356 ID

ELI 5  $\mu\text{g}/\text{mL}$  357

ELI 20  $\mu\text{g}/\text{mL}$  358

ELI 100  $\mu\text{g}/\text{mL}$  359

- SKov-3 Unt 360

5  $\mu\text{g}/\text{mL}$  361

20  $\mu\text{g}/\text{mL}$  362

100  $\mu\text{g}/\text{mL}$  363

- TOV1120 Unt 364

5  $\mu\text{g}/\text{mL}$  365

20  $\mu\text{g}/\text{mL}$  366

100  $\mu\text{g}/\text{mL}$  367

- A2780 Unt 368

5  $\mu\text{g}/\text{mL}$  369

20  $\mu\text{g}/\text{mL}$  370

100  $\mu\text{g}/\text{mL}$  371

- OVA Unt 372

5  $\mu\text{g}/\text{mL}$  373

20  $\mu\text{g}/\text{mL}$  374

100  $\mu\text{g}/\text{mL}$  375

FT 33 Unt 376

No talc Unt 377

5  $\mu\text{g}/\text{mL}$  378

20  $\mu\text{g}/\text{mL}$  379

100  $\mu\text{g}/\text{mL}$  380

1000  $\mu\text{g}/\text{mL}$  381

Control -5 377

Control 1000 378

ELI Control -5 379

Control 1000 380

1000  $\mu\text{g}/\text{mL}$  talc 381

SKov-3 Control -5 382

Control 1000 383

1000  $\mu\text{g}/\text{mL}$  talc 384

A2780 Control -5 385

Control 1000 386

1000  $\mu\text{g}/\text{mL}$  talc 387

Numer of Epi - Unt 388

5  $\mu\text{g}/\text{mL}$  389

20  $\mu\text{g}/\text{mL}$  390

100  $\mu\text{g}/\text{mL}$  391

1000  $\mu\text{g}/\text{mL}$  talc 392

control -5 393

Control 1000 394

TOV1120 Control -5 395

Control 1000 396

1000  $\mu\text{g}/\text{mL}$  talc 397

Prepare talc

100mg in 10mL  $\rightarrow$  mix and filter = 10mg/mL

2/2/18 treated w/talc

$$(x)(10000 \text{ ug/mL}) = (5 \text{ mL})(5 \text{ ug/mL}) = 2.5 \text{ mL}$$

$$(x)(10000 \text{ ug/mL}) = (5 \text{ mL})(20 \text{ ug/mL}) = 10 \text{ mL}$$

$$(x)(10000 \text{ ug/mL}) = (5 \text{ mL})(100 \text{ ug/mL}) = 50 \text{ mL}$$

2/6/18

treat w/ "soaked" talc (10000 ug/mL)

\* talc was rocked for 72 hours, spun and supernatant collected

★ too much volume

made 1g/10 mL DMSO - Re-soak 72hr

2/7/18 seeded Normal DN. Epi Cells  $1 \times 10^6$   $\rightarrow$  treat Friday 2/9/18 ✓

2/26/18

383 NOE

0 ug/mL	383
5 ug/mL	384
20 ug/mL	385
100 ug/mL	386

Monday 2/11/18

2/16/18

RNA

41053

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	356	2/5/2018 1:18:50 PM	0.0830	µg/µl	2.074	1.109	1.87	1.30	RNA	40.00
2	357	2/5/2018 1:19:20 PM	0.1000	µg/µl	2.500	1.342	1.86	1.18	RNA	40.00
3	358	2/5/2018 1:19:39 PM	0.0829	µg/µl	2.073	1.118	1.85	1.26	RNA	40.00
4	359	2/5/2018 1:20:00 PM	0.0349	µg/µl	0.873	0.476	1.84	0.39	RNA	40.00
5	360	2/5/2018 1:20:24 PM	0.2387	µg/µl	5.968	2.966	2.01	0.78	RNA	40.00
6	361	2/5/2018 1:20:43 PM	0.3389	µg/µl	8.473	4.194	2.02	1.15	RNA	40.00
7	362	2/5/2018 1:21:04 PM	0.3017	µg/µl	7.542	3.837	1.97	1.47	RNA	40.00
8	363	2/5/2018 1:21:20 PM	0.1118	µg/µl	2.796	1.465	1.91	1.53	RNA	40.00
9	368	2/5/2018 1:21:41 PM	0.2203	µg/µl	5.508	2.880	1.91	1.34	RNA	40.00
10	369	2/5/2018 1:21:57 PM	0.2474	µg/µl	6.185	3.187	1.94	2.03	RNA	40.00
11	370	2/5/2018 1:22:12 PM	0.2217	µg/µl	5.541	2.855	1.94	1.63	RNA	40.00
12	371	2/5/2018 1:22:29 PM	0.1336	µg/µl	3.340	1.726	1.93	1.42	RNA	40.00

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	379	2/16/2018 9:27:37 AM	0.1685	µg/µl	4.212	2.034	2.07	1.01	RNA	40.00
2	380	2/16/2018 9:27:55 AM	0.0658	µg/µl	1.645	0.713	2.31	3.02	RNA	40.00
3	381	2/16/2018 9:28:13 AM	0.0801	µg/µl	2.003	0.891	2.25	0.96	RNA	40.00
4	382	2/16/2018 9:28:30 AM	0.3084	µg/µl	7.711	3.759	2.05	2.24	RNA	40.00
5	383	2/16/2018 9:28:51 AM	0.2921	µg/µl	7.303	3.582	2.04	1.09	RNA	40.00
6	384	2/16/2018 9:29:10 AM	0.1812	µg/µl	4.531	2.179	2.08	2.15	RNA	40.00
7	385	2/16/2018 9:29:29 AM	0.0869	µg/µl	2.172	0.971	2.24	1.31	RNA	40.00
8	386	2/16/2018 9:29:51 AM	0.0116	µg/µl	0.289	-0.017	-16.61	-5.65	RNA	40.00
9	387	2/16/2018 9:30:11 AM	0.0133	µg/µl	0.332	0.013	25.53	-15.74	RNA	40.00
10	395	2/16/2018 9:30:29 AM	0.2169	µg/µl	5.421	2.598	2.09	0.65	RNA	40.00
11	397	2/16/2018 9:30:51 AM	0.1328	µg/µl	3.321	1.575	2.11	2.26	RNA	40.00
12	396	2/16/2018 9:31:08 AM	0.1633	µg/µl	4.084	1.949	2.10	2.47	RNA	40.00

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
16	364	2/16/2018 9:49:26 AM	0.2401	µg/µl	6.003	2.879	2.09	1.78	RNA	40.00
17	365	2/16/2018 9:49:46 AM	0.2418	µg/µl	6.044	2.939	2.06	1.27	RNA	40.00
18	366	2/16/2018 9:50:01 AM	0.2043	µg/µl	5.106	2.459	2.08	1.88	RNA	40.00
19	367	2/16/2018 9:50:16 AM	0.1712	µg/µl	4.281	2.026	2.11	1.83	RNA	40.00

41054

cDNA

21/6/18

- supers VIVO  
kit

## 0.5 ug RNA Reaction

ID	ul RNA	ul water
356	6.0	10.0
357	5.0	11.0
358	6.0	10.0
359	14.3	1.7
360	2.1	13.9
361	1.5	14.5
362	1.7	14.3
363	4.5	11.5
364	2.1	13.9
365	2.1	13.9
366	2.4	13.6
367	2.9	13.1
368	2.3	13.7
369	2.0	14.0
370	2.3	13.7
371	3.7	12.3
379	3.0	13.0
380	7.6	8.4
381	6.2	9.8
382	1.6	14.4
383	1.7	14.3
384	2.8	13.2
385	5.8	10.2
386	16.0	0.0
387	16.0	0.0
395	2.3	13.7
397	3.8	12.2
396	3.1	12.9

